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Applicant: SUMIDA ET AL. Examiner: B.J. FETTEROLF  
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MEASUREMENT

CERTIFICATE UNDER 37 CFR 1.6(a):

I hereby certify that this paper is being transmitted by facsimile to the U.S. Patent and Trademark Office on December 27, 2006.

By:  
Name

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DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Kyoichi Sumida, hereby declare as follows.

I graduated from Hiroshima University in March, 1995.

I began employment with Wako Pure Chemical Industries, Ltd., the Assignee of the above-identified application in April, 1995 and have been engaged in said company, since that time, in the study of immunological reagent and assay thereof in Osaka Research Laboratories.

I am co-inventor of the above-identified the application and am well aware of the September 27, 2006 Office Action.

The following experiments were conducted by me.

Experimental data -Measurement of blank values using various polymers as an agglutination accelerator -

(1) Preparation of an anti-human PSA antibody sensitized latex test solution

A mixture of 0.5 ml of 50 mM borate buffer (pH 7.1) containing 0.7 mg of anti-human PSA mouse monoclonal antibody (made by Wako Pure Chemical Industries, Ltd.) and 0.5 ml of 50 mM borate buffer (pH 7.1) containing a polystyrene latex (particle size 0.28  $\mu$ m, made by Sekisui Chem. Co. Ltd.) suspended in 2% by W/V, was reacted at 25 °C for 2 hours. Subsequently, the latex separated by centrifugation was washed with 50 mM borate buffer (pH 7.1), and said latex was suspended in 50 mM borate buffer (pH 7.3) containing 0.5% by W/V of BSA so that the concentration of the latex became 0.1% by W/V, and the thus obtained mixture was used as an anti-human PSA antibody sensitized (immobilized) latex test solution.

(2) Sample

A solution of 10 mM phosphate buffer (0.85% NaCl) containing 1.0% by W/V of BSA was used as a sample.

(3) Reagents

i) Test solution No. 1

A solution of 100 mM HEPES-NaOH buffer (pH 7.0) containing 1% of one of the following polymer as an agglutination accelerator, 0.5% of BSA and 1% of NaCl was used as test solution No. 1.

Polymer1: Copolymer of 2-methacryloyloxyethylphosphorylcholine / n-butyl methacrylate (the ratio of monomer = 8 / 2)

Polymer5: Copolymer of 2-methacryloyloxyethylphosphorylcholine / benzyl methacrylate (the ratio of monomer = 8 / 2)

Polymer6: Homopolymer of 2-methacryloyloxyethylphosphorylcholine

ii) Test solution No. 2

The anti-human PSA antibody sensitized (immobilized) latex test solution prepared in (1) was used as a test solution No. 2.

(4) Assay method

Assay was performed under the following measuring conditions by using a BM-8 automatic analyzer made by JEOL Ltd. The assay was repeated 5 times.

Sample: 10  $\mu$ l

Test solution No. 1: 90  $\mu$ l

Test solution No. 2: 30  $\mu$ l

Assay method: 2-point end method (84 point and 65 point)

Main wavelength: 694 nm

## (5) Results

(ng/ml)	Polymer5	Polymer1	Polymer6
1 <sup>st</sup>	4	5	12
2 <sup>nd</sup>	3	8	-4
3 <sup>rd</sup>	-4	-8	-11
4 <sup>th</sup>	-8	5	6
5 <sup>th</sup>	2	-5	-5
Variation	8	18	23

As is clear from the results, blank value obtained by using the Polymer1 shows a level of variation about 1.5 times that obtained by using the Polymer5. Blank value obtained by using the Polymer6 shows a level of variation about 3 times that obtained by using the Polymer5. Thus, it is found that Polymer5 is a superior agglutination accelerator which has little influence on blank value.

I, the undersigned declarant, declare further under the penalty of perjury of the laws of the United States that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Signed this December 27, 2006

Kyoichi Sumida

52835  
PATENT TRADEMARK OFFICE

Kyoichi Sumida  
Signature

## Review article

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## PROSTATE-SPECIFIC ANTIGEN AS A TUMOR MARKER IN PROSTATE CANCER

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Key words: prostate-specific antigen, tumor marker, prostate cancer, tissue-specific antigens, early detection, monitoring

## INTRODUCTION

Prostate cancer has become one of the leading malignant diseases in males in the United States,<sup>1-3</sup> being the most frequent in terms of incidence and second in terms of mortality. Even in Japan, the number of patients with prostate cancer is increasing and more than 2 in 100,000 inhabitants die annually from it. The cancer displays some special characteristics such as a racial difference in the incidence of the clinical cancer,<sup>4</sup> a slow development in some cases, good response to androgen therapy in about 80% of cases and a lack of suitable therapy after relapse.<sup>4-5</sup>

Numerous efforts have been made to reduce the mortality due to this cancer.<sup>4,6-11</sup> To this end, the early detection of prostate cancer, and adequate monitoring during treatment and detection of relapse or recurrence have been thought to be very important. At the present time, early detection of prostate cancer is not necessarily associated with a good prognosis<sup>12-15</sup> although the distribution of stages, which is believed to be the major risk factor of prostate cancer, differs between those cases found by mass screening and symptomatic ones.<sup>16</sup>

To diagnose and monitor prostate cancer, acid phosphatase [orthophosphoric monoester phosphohydrolase, EC 3.1.3.2., (AP)] as well as digital rectal examination (DRE) are routinely used. The significance of AP as a tumor marker of prostate cancer was first described by Outman et al. in 1936,<sup>17</sup> who found a high correlation between serum AP activity and the occurrence of prostate cancer, especially in the metastasized stages to bones. As AP is widely distributed in red blood cells, leukocytes, platelets, and organs such as liver, spleen and kidneys as well as prostate, diseases other than prostate cancer may also cause elevated serum AP activity. Therefore, low specificity is the major problem associated with the use of AP as a tumor marker. In 1957, Schulman et al.<sup>18</sup> purified a specific fraction of AP from prostate tissues (prostatic AP, PAP) and performed a clinical

evaluation using single radial immunodiffusion. Following this, several immunoassays for the detection of PAP were developed by Cooper and Foti,<sup>19</sup> Vihko et al.,<sup>20</sup> Chu et al.,<sup>21</sup> Choe et al.,<sup>22</sup> and Lee et al.<sup>23</sup> Today, PAP is usually assayed by radioimmunoassay (RIA)<sup>24</sup> or enzyme immunoassay (EIA).<sup>25</sup> The clinical evaluation of PAP assays has resulted in a highly specific and low sensitive technique, especially in the early stages of prostate cancer.<sup>26-28</sup> Therefore, when the clinician finds a high serum PAP in a patient, it strongly suggests advanced prostate cancer. However, if a patient has a normal serum PAP level, prostate cancer cannot be excluded.<sup>27</sup>

Another tumor marker of prostate cancer, distinct from PAP, has also been studied for a long time. Prostate-specific antigen (PSA) was identified and purified to homogeneity from human prostate by Wang et al. in 1979.<sup>29</sup> Since then, PSA has been studied by many investigators all over the world and its superiority as a tumor marker of prostate cancer has been demonstrated. So much so that it is now one of the best tumor markers available.

In this review, we describe the identification, biochemical characterization and clinical significance of PSA. Recent advances involving PSA density and the PSA-protease inhibitor complex are also discussed. Finally, the standardization of PSA assays is examined. Many excellent reviews of this field have been published mainly in the United States and Europe,<sup>30-42</sup> and here the achievements of Japanese researchers are highlighted.

## IDENTIFICATION, PURIFICATION, AND BIOCHEMICAL PROPERTIES OF PSA

Since 1960, many efforts to find tumor markers of prostate cancer other than PAP have been made by Flocks et al.,<sup>43</sup> Ablin et al.,<sup>44</sup> Li et al.<sup>45</sup> and Sensabaugh.<sup>46</sup> Wang et al. of Roswell Park Memorial Institute, USA, (RPMI) were the first to identify and purify to homogeneity a glycoprotein immunologically distinct from PAP and named it prostate-specific antigen (PA); in 1979 the name was changed to PSA.<sup>29</sup> In their paper, they reported that the concentration of PSA in benign prostatic hyperplasia

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(BPH) tissue was 95 µg/g and 700 µg/ml in seminal plasma. Both purified PSAs were found to be immunologically identical and have similar biochemical properties.<sup>47</sup> Following that, Papsidero et al.<sup>48</sup> of the same department identified PSA in human serum and confirmed that this material was identical to that purified directly from prostatic tissue. This suggested that PSA could be used as a sero-marker of prostate cancer.

PSA is a glycoprotein containing about 10% carbohydrate and its molecular weight is 33,000, determined by Sephadex G-75 (Pharmacia Biotech AB, Uppsala, Sweden) column chromatography. Upon SDS-polyacrylamide gel electrophoresis, purified PSA gives a single band with a location corresponding to a molecular weight of 34,000, indicating that it contains a single polypeptide. Sucrose density gradient ultracentrifugation showed a single protein band with a sedimentation coefficient of 3.1 S. The isoelectric point of the purified preparation was found to be approximately 6.9.<sup>39,47</sup> PSA has 240-amino acid residues and 4 carbohydrate side-chains. The N-terminal amino acid is isoleucine and the C-terminal residue is proline. The N-linked carbohydrate side-chain is at amino acid 45; asparagine and the O-linked carbohydrate side-chains are at amino acid 69, serine, at 70, threonine and at 71, serine.<sup>49</sup> The complete gene encoding PSA has been sequenced and localized to chromosome 19. The gene was found to be approximately 6 kb in size and composed of 4 introns and 5 exons.<sup>50</sup> PSA and the proteases of the kallikrein family of genes were found to exhibit a high degree of homology.<sup>51</sup> PSA is a kallikrein-like serine protease that is produced exclusively by the epithelial cells lining the acini and ducts of the prostatic gland. PSA is secreted into the lumina of the prostatic ducts and is present in seminal plasma. In seminal fluid, PSA is

involved directly in the liquefaction of the seminal coagulum that is formed at ejaculation.<sup>52</sup> As far as specific enzymatic activity is concerned, Ban found it to possess chymotrypsin (ACT)- and trypsin-like activity.<sup>53</sup> These observations led to the recent use of serum PSA-ACT or free PSA determinations for the differential diagnosis of prostate cancer from BPH, to be discussed later. The protein,  $\gamma$ -seminal protein ( $\gamma$ -Sm), which was the first prostate-specific antigen to be studied, was purified by Hara et al.<sup>54</sup> and has been mainly evaluated in Japan.<sup>55-58</sup> This and p30 identified by Sensabaugh are thought to be identical immunologically and functionally to PSA.<sup>46,59-60</sup> Seminal vesicle-specific antigen (SVSA) is also thought to be the substrate for PSA during semen liquefaction.<sup>60</sup> The characteristics of PSA are summarized in Table 1 and compared with those of PAP and  $\gamma$ -Sm.

#### CLINICAL CHARACTERISTICS OF THE PSA ASSAY

Serum PSA was first measured by researchers at the RPMI. Papsidero et al.<sup>48</sup> were able to measure PSA in 17 of 219 patients with advanced prostate cancer using rocket immunoelectrophoresis. Then Kuriyama et al.<sup>61</sup> developed a highly sensitive enzyme immunoassay (EIA, sensitivity, 0.10 ng of PSA/ml serum) and studied PSA levels using serum samples collected during the National Prostatic Cancer Project in the USA. They found that the mean serum PSA of 51 normal men was  $0.47 \pm 0.66$  ng/ml. When a value of 1.8 ng/ml (mean + 2SD) was taken as the upper limit of normal, it was found that 68% of BPH cases had an elevated PSA. So too did 63% of stage A prostate cancers, 79% of stage B, 77% of stage C and 86% of stage D. This report is the first detailed clinical evaluation of PSA.

Table 1. Physico chemical characteristics of PSA compared with  $\gamma$ -Sm and PAP.

	PSA	$\gamma$ -Sm	PAP
Characteristics	Glycoprotein	←	←
Molecular weight	33,000-34,000	23,000, 33,000	100,000
Subunit	Monomer	←	Dimer
Isoelectric point	6.9	5.8-7.1	4.2-5.7
Sedimentation coefficient	3.1 S	2.55 S	5.7 S
Carbohydrate content	10%	12%	7%
Ammonium sulfate precipitation	45-50%	←	50-70%
Amino acid Number	237, 240	237	784
N-terminal	Aspartate	Aspartate	Arginine
Optimum pH	7.0	←	4.8-6.0
Cell localization in prostate	Epithelial cell	←	←
Secretory nature	Present	←	←
Biological half-life	2.4 d		1.2 h
Function	Serine protease	←	Phosphatase

## PSA in prostate cancer

As Osterling mentioned in his review,<sup>40</sup> it is important for the reader to remember which assay system is used to determine PSA when its various clinical characteristics are discussed. The first available commercially PSA assay kit was distributed by Hybritec, San Diego, USA in 1984<sup>42</sup> (Tandem-R PSA), then Yang et al. developed another radioimmunoassay (RIA) in 1985 (Pro-Check PSA; Yang Laboratory, Bellevue, USA).<sup>43</sup> Both kits are made in the USA and have been widely used there and in Europe. In Japan, Miki et al.<sup>44</sup> evaluated Eiken PSA (RIA) (Eiken Chemical Co., Ltd., Tokyo, Japan) in 1984 and Kuriyama et al.<sup>45</sup> assessed an EIA (Markit-F PA; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) for the determination of PSA in 1987. Japanese urologists, therefore, are used to Eiken PSA or Markit-F PA assays. Some differences in the clinical data have been found when PSA evaluations have been carried out. Recently, more than 10 different assay kits have become available in Japan which has caused some confusion in evaluating the clinical data.

Stamey et al.<sup>34</sup> and Osterling et al.<sup>46</sup> simultaneously and independently calculated the half-life of serum PSA. Using Pro-Check PSA, Stamey et al. determined the half-life to be  $2.2 \pm 0.8$  days, whereas Osterling et al. obtained a slightly longer half-life of  $3.2 \pm 0.1$  days using the Tandem-R PSA assay. Compared with PAP, and irrespective of its relatively small molecular weight, PSA has a long half-life. Both groups found it necessary to wait for 2-3 weeks to obtain a baseline level of serum PSA after prostatic manipulation such as occurs during transrectal ultrasound, biopsy, and various treatments involving the prostate.

Serum PSA concentrations do not appear to fluctuate unpredictably or display a circadian rhythm during a 24-hour period, compared with PAP, following the observations of Dejer et al.<sup>67</sup> and Maatman.<sup>68</sup> Since serum PSA values change very little during any 24-hour period, there does not appear to be an optimum time for its measurement. Stamey et al.<sup>34</sup> found that serum PSA decreases 18% on average, 24 h after hospitalization, but the exact reason for this is not yet understood.

The effect of DRE has been studied as one of a number of factors affecting serum PSA determination.<sup>69-70</sup> Neither PSA nor PAP levels changed significantly 5 to 39 min after DRE, as shown by Brawer et al.<sup>70</sup> In contrast, Stamey noted a 1.92-fold increase in post-DRE serum PSA levels.<sup>71</sup> He also found a 4-fold increase due to cystoscopy and a 57-fold elevation after needle core biopsy of the prostate.<sup>71</sup> Transrectal ultrasonography alone, however, appeared to have very little effect on serum PSA.<sup>72</sup> Falseley elevated serum PSA concentrations, due to prostatitis and urinary retention, are well recognized.<sup>73-76</sup> Stamey found

that transurethral resection of the prostate causes a 53-fold increase in the immediate post-operative PSA serum levels.<sup>71</sup> However it should be remembered that these findings were obtained using the Tandem-R PSA or Pro-Check PSA assay kits. Due to lack of data from a relatively large number of patients, the effects of these factors in Japanese patients investigated using Eiken PSA or Markit-F PA kits still require confirmation.

## CLINICAL IMPORTANCE OF PSA IN THE DETECTION OF PROSTATE CANCER

Following the initial clinical investigation performed by researchers at the RPMI, clinical studies to assess the significance of PSA were begun. Kuriyama et al. of RPMI,<sup>41</sup> using their original EIA, were unable to detect PSA in sera from female patients with various types of cancer. The mean serum PSA and its standard deviation in normal males and males with non-prostatic cancers were similar. As mentioned above, 68% of BPH cases exhibited elevated PSA values, which implies a low specificity for PSA in the detection of prostate cancer. However, there was a statistically significant difference between the serum PSA values in BPH and prostate cancer. Kuriyama et al. showed that simultaneous determination of PSA and PAP offers an increased sensitivity (51% PSA in a single assay compared with 80% in combination) and high specificity (90%) for the diagnosis of prostate cancer. As a cut-off value for PSA, they used a mean PSA + 2SD in BPH cases.<sup>77</sup> Following the observation of Papsidero et al.<sup>48</sup> that the molecular weight of PSA in sera was about 100,000, Chu and Kuriyama found a binding protein for PSA in the sera of patients with prostate cancer. This protein was purified by affinity chromatography and found to belong to the IgG<sub>2</sub> family.<sup>78</sup> Patients with stage D prostate cancer have been shown to have significantly high levels of binding protein using reversed EIA for the PSA determination.<sup>79</sup> This protein was later found to be ACT.

Using Tandem-R PSA or Pro-Check PSA assays, numerous reports have been published evaluating serum PSA as a means of screening or diagnosing prostate cancer.<sup>62-63,69,73,80-96</sup> Recently, a new EIA has been developed in the USA (IMx Dainapack, Abbott, Chicago, USA) and evaluated there and in Japan.<sup>97-98</sup> Japanese researchers have also published many studies assessing the clinical evaluation of PSA, mainly using Eiken PSA, Markit-F PA and other assay kits.<sup>64-65,99-122</sup>

Normal values in males of 4.0 ng/ml and 2.5 ng/ml have been established for the Tandem-R PSA<sup>123</sup> and Pro-Check PSA assays,<sup>34,123</sup> respectively. Of the healthy males studied, 97% had PSA values <4.0 ng/ml in the Tandem-R PSA assay and the normal value

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in the Pros-Check assay was taken to be the mean + 2SD (about 95% of patients). However, since it is well known that serum PSA values measured by the Pros-Check assay are usually higher than those obtained by the Tandem-R assay, these "reversed" normal values are strange. When we evaluated serum PSA in normal Japanese age-matched males (age: > 50 years), we obtained for the mean + 3SD of the serum PSA a value of 1.9 ng/ml in the Tandem-R PSA assay<sup>124</sup> and 3.2 ng/ml in the Pros-Check PSA assay.<sup>117</sup> In addition, the normal value of serum PSA in Japanese and European patients is similar using the Delfia PSA assay kit (Pharmacia Biotech AB, Uppsala, Sweden).<sup>118</sup> Thus, for any discussion or decision involving the normal value of serum PSA, racial differences as well as assay methods need to be considered. Irrespective of which assay method or normal value is used, at least 1-5% of asymptomatic males have an elevated serum PSA because PSA is an antigen which is prostate tissue-specific but not cancer-specific.

In clinical practice, distinction between BPH and prostate cancer is very important when using PSA as a tumor marker. Table 2 summarizes the positive rates obtained with serum PSA for these 2 groups of patients, from data gathered from a relatively large number of studies performed in the USA and Japan.<sup>85,96,117-119,120,124-127</sup> As shown in Table 2, if normal values were used as cut-off points, 24.5-75.0% of BPH cases exhibit elevated serum PSA levels. In addition, the differences in the positive rates, shown by the PSA determinations, between BPH and stage A or B prostate cancer are variable: 6-36.9%. The positive rates in the advanced stages were almost 100%. Thus, serum PSA increases with the progression of both the clinical and pathological stages. Compared with the results obtained in the USA, prostate cancer is characterized in Japan by the low specificity (high-false posi-

tive rate for BPH) and relatively high sensitivity for diagnosis for organ-confirmed stages.<sup>6</sup> This may be due to the lower cut-off point chosen.

As a cause of false positives in BPH cases other than the characteristics of the PSA tissue-specificity, the volume of prostatic tissue is thought to play a part, reflecting serum PSA values.<sup>34,107,117</sup> Stamey et al.<sup>34</sup> have calculated that benign hyperplastic tissue elevates the serum PSA level at a rate of 0.3 ng/ml/gm BPH tissue (Pros-Check PSA). However, Weber et al.<sup>128</sup> were unable to demonstrate such a close relationship between the amount of BPH tissue and serum PSA concentration and observed a glandular component with PSA giving rise to varying serum PSA levels. Another factor contributing to elevated serum PSA in cases of BPH is thought to be coexisting prostatic intraepithelial neoplasia. Brawer<sup>41</sup> and Lee et al.<sup>129</sup> found a proportional increase in serum PSA with coexisting intraepithelial neoplasia.

As another cause of overlapping serum levels of PSA between patients with BPH and those in the early stages of prostate cancer, the characteristics of PSA assay systems themselves must be considered: detection limits, standards used, antigen sites of each anti-PSA antibody, among others. Indeed, Horton et al.<sup>130</sup> and Akinoto et al.<sup>109</sup> have found that differences in clinical data between the Tandem-R PSA and Pros-Check PSA assays depend upon a difference in PSA expression by the standard. Moreover, Arai et al.<sup>131</sup> demonstrated that lymph node involvement could be suggested by a combination of serum PSA values (Markit-F PA) and Gleason score. This finding was not confirmed by the determination of PSA with the Tandem-R PSA assay. Table 3 shows the serum PSA values used to obtain various degrees of specificity, with the use of BPH cases as controls. This was per-

*Table 2. Serum PSA levels in patients with BPH and localized prostate cancer.*

Assay methods	Cut-off value	No of cases				Positive rate (%)	
		BPH	Prostate cancer		BPH	Prostate cancer	
			Stages A/B	Stage C		Stages A/B	Stage C
Tandem-R PSA <sup>a</sup>	4.0	597	319		24.5	57.4	
Markit-F PA	3.8	111	33	22	26.1	63	95
Elken PA	3.0	116	22	21	50.8	68	100
Pros-Check PSA	3.2	132	27	23	75.0	83	98
Delfia PSA	2.0	126	33	30	66.7	79	97
Ball ELSA	2.0	125	33	30	67.2	79	97
ACS-PSA	2.3	68	19	11	62	68	91
Tandem-R PSA	1.9	149	29	28	68.4	83	96
Markit-M PA	1.8	137	32	22	34.3	67	93
IMx PA	4.0	112	32	46	41.1	81	93

<sup>a</sup> Summaries of the data from Lange, Hudson and Parlin (Ref #92, 125, 126).

## PSA in prostate cancer

Table 3. Serum PSA levels determined with various degrees of specificity.

Specificity (%)	PSA assay systems: PSA (ng/ml)							
	Markit-F	Eiken	Tandem-R	Pros-Check	Delfia	Ball ELSA	ACS	Markit-M
90	5.5	8.1	21	32	15	18	17	4.5
80	4.2	5.9	9.0	19	9.2	11	9.5	2.9
70	3.4	4.3	6.2	12	6.3	6.7	7.9	2.0
60	2.8	3.6	4.6	8.9	4.9	5.4	5.3	1.2
50	2.1	3.0	3.6	5.9	3.7	3.5	3.8	0.9

formed using almost the same Japanese patients as in the investigation of the different assays. Compared with the normal value in each assay system listed in Table 2, the serum PSA values exhibit a high degree of specificity. However, some kits such as Markit-F, Eiken PSA and Markit-M which were originally made in Japan do not show such a marked specificity (80 or 90%) compared with the normal value. In addition, another suitable cut-off value for the differential diagnosis of prostate cancer from BPH needs to be established. The best results were obtained with values of 9-13 ng/ml in the Tandem-R PSA assay<sup>124</sup> as found in the USA<sup>125</sup> and 3.6 ng/ml in the Markit-M PA assay.<sup>120</sup> Sensitivities for the diagnosis of stages A/B and C prostate cancer are presented in terms of various degrees of specificity in Table 4. Thus, using PSA alone, the detection or screening for the early stages of prostate cancer with a high degree of specificity is still limited.

Although, as initial studies recognized,<sup>77</sup> the simultaneous determination of PSA and PAP has very little effect in improving the efficacy of prostate cancer detection,<sup>117</sup> only in a few cases of prostate cancer is the PSA negative and PAP positive. In an attempt to increase the clinical efficiency of the diagnosis of pros-

tate cancer, Cooner et al.<sup>133</sup> found a combination of PSA detection and DRE to be useful.

The clinical significance of serum PSA for the diagnosis of prostate cancer can be summarized as follows: (1) Normal values differ for each assay method; (2) Prostate cancer patients have significantly elevated serum PSA levels which increase as the disease progresses both clinically and pathologically; (3) Some cases of BPH also exhibit mild to moderately high serum PSA levels due to the prostate volume or coexisting intracryptal neoplasia; (4) To obtain an effective diagnosis, cut-off points need to be modified used in combination with DRE; and (5) As mentioned by Chybowski et al.<sup>135</sup> and Aral et al.,<sup>131</sup> serum PSA measurement reduces the need for bone scintigraphy or helps in the recognition of lymph node involvement. Although there are some limitations and careful analysis of the data is necessary, it is clear that PSA measurement is the best tool for the diagnosis of prostate cancer we have, compared with other methods such as DRE and transrectal ultrasonography. However, we must have sufficient information about the characteristics of the assay used to determine PSA, before a meaningful analysis of the data can be made.

Table 4. Sensitivities of PSA determinations at various degrees of specificity.

Specificity (%)	Stages in prostate cancer	PSA assay systems; sensitivities (%)							
		Markit-F	Eiken	Tandem-R	Pros-Check	Delfia	Ball ELSA	ACS	Markit-M
90	A + B	44	27	28	26	36	30	32	39
	C	82	86	76	78	83	83	91	79
80	A + B	58	41	45	37	42	45	53	56
	C	95	95	75	87	90	90	91	90
70	A + B	68	45	59	44	58	55	53	61
	C	95	95	89	87	93	90	91	93
60	A + B	72	55	59	52	58	58	58	69
	C	95	95	89	87	93	93	91	95
50	A + B	81	68	71	59	64	67	58	78
	C	95	100	89	87	97	97	91	95



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# PSA AS A FOLLOW-UP MARKER IN PATIENTS WITH PROSTATE CANCER

The initial investigations were performed at the RPMI. Kuriyama et al.<sup>134</sup> demonstrated that serum PSA levels during the pre-chemotherapeutic period correlated with prognosis in 96 patients with advanced prostate cancer who died during the observation period. In addition, there was a trend towards longer survival in those patients with lower initial PSA values. The possible value of serum PSA was established as a means of monitoring prostate cancer in 19 patients with metastatic disease and 34 patients who had received curative therapy for localized disease. Of the 19 prostate cancer patients, 14 (74%) cases involved PSA analysis of 232 samples and the clinicopathological evaluation of treatment response. In another study, a total of 161 serum samples were analyzed for PSA during a period of 12–114 weeks. Five patients developed metastasis and all exhibited increasingly elevated PSA levels. An elevated serum PSA was detected 68 weeks before the recurrence of the disease was clinically diagnosed in 1 patient.<sup>134–136</sup>

In the USA and Europe, clinical evaluations of serum PSA as a follow-up marker have been carried out.<sup>49,137–158</sup> Generally speaking, serial PSA levels were found to be the best reflection of disease status. For example, PSA levels were elevated at relapse or progression, fell or returned to normal during successful treatment, and rose in the presence of a residual tumor or unsuccessful therapy, irrespective of the nature of the treatment such as total prostatectomy,<sup>141,147–148,150</sup> irradiation therapy,<sup>142,144,149,150</sup> and endocrine treatment.<sup>139–140,145,148,151–153,155,158</sup> Serum PSA could be detected several weeks after radical prostatectomy due to its long half-life.<sup>126</sup> After becoming undetectable, if the PSA rose to detectable levels again, 100% of such patients relapsed 3–6 months later.<sup>126</sup> A correlation between high or increasing serum PSA levels and disease progression was found in radiation-treated patients by Stamey et al.<sup>142</sup> and Kaplan et al.<sup>150</sup> However, as noted by Goldrath and Messing and Leo et al., it must be remembered that there are cases where the serum PSA levels do not increase on disease progression<sup>147</sup> or where PSA has not identified some cases with stage D2 prostate cancer, treated by hormonal therapy.<sup>177</sup>

As in the USA and Europe, there are several Japanese reports evaluating PSA as a tool for follow-up during anti-androgen therapy.<sup>112,117–118,120,131,159–161</sup> For example, Akinoto et al.<sup>160</sup> found no relationship between patient prognosis and the serum PSA level before initiation of treatment. However, when the patients were divided into 2 groups, those who had normal levels of serum PSA 1–6 months after treat-

ment and those who did not, there was a statistically significant difference in prognosis between the groups. The authors also found a statistically significant correlation between the patient prognosis and the minimum level of serum PSA observed. Shinoda<sup>161</sup> observed the serum PSA fall to about half the pre-treatment value 7 days after initiation of successful endocrine therapy. Then he divided his patients into 2 groups, those who did or did not achieve a level of less than 50% of their initial serum PSA at 7 days, and found a statistically significant correlation with no progression of disease (Fig. 1). This data suggests that serial serum PSA levels are strongly correlated with the clinical response and are helpful for establishing the prognosis of prostate cancer patients treated with endocrine therapy. Recently, there have been some reports in which serum PSA changed in parallel with serum testosterone levels.<sup>156–157,162</sup> Therefore, in assessing the significance of serum PSA levels in patients treated with endocrine therapy, such effects need to be taken into account. Almost all patients relapsing or progressing had elevated PSA levels.<sup>117–118,120,131,159–161</sup> Kuriyama et al.<sup>137</sup> have proposed criteria for evaluating prostatic tumor markers during follow-up (Table 5). Using these criteria, the clinical usefulness of various assay kits for PSA,  $\gamma$ -Sm

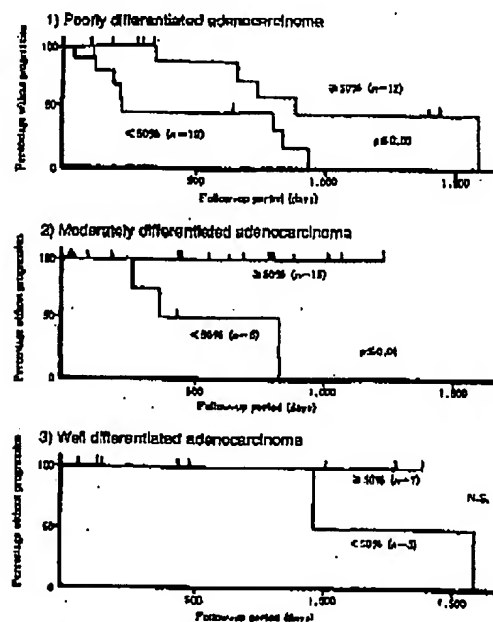


Fig. 1. Percentage without progression, according to the reduction in serum PSA on the 7th day.

## PSA in prostate cancer

Table 5. Criteria for evaluating tumor markers in patients with prostate cancer on follow-up.

Response	Responders (CR, PR & S)	Nonresponders (PD)
Score 4	Abnormal → Normalizing Abnormal → Decreasing > 1/2 within 7 days	Normal → Abnormalizing before diagnosis
3	Abnormal → Decreasing > 1/2	Normal → Abnormalizing at diagnosis Abnormal → Increasing
2	Abnormal → Decreasing < 1/2 Normal → Normal	Abnormal → Abnormal Normal → Abnormalizing after diagnosis
1	Abnormal → No decrease Normal → Increasing	Abnormal → Decreasing Normal → Normal

and PAP was evaluated (Table 6).<sup>124</sup> Matching rates (score;  $\geq 3$ ) and mean scores were higher for PSA than those for  $\gamma$ -Sm or PAP, again objectively confirming the superiority of PSA.

## PSA AS AN IMMUNOHISTOCHEMICAL MARKER OF PROSTATE NEOPLASM

Nadi et al.<sup>163</sup> in the USA and Okano et al.<sup>164</sup> in Japan using the same antibody (p17)<sup>20</sup> from the same source (RPMI) performed immunohistochemical studies on PSA. Based upon data obtained in later studies,<sup>165-169</sup> 99% of both primary and metastatic prostatic lesions stained positive for PSA while the corresponding figure in non-prostatic tissue was 0%. Therefore, PSA staining could be used to detect adenocarcinoma metastasized from an unknown origin.<sup>107,160-170</sup> More-

over, Deguchi et al.<sup>171</sup> detected micrometastasis to lymph node and bone by applying the polymerase chain reaction technique to PSA. A limitation of the immunohistochemical staining of PSA was the finding of weak staining in poorly differentiated carcinoma<sup>163,169,172</sup> in spite of other evidence<sup>167</sup> and some slight cross-reactivity with tissues other than the prostate.<sup>173</sup> As far as a correlation between PSA staining and disease progression is concerned, Epstein and Eggelston<sup>166</sup> found only a small area staining weakly for PSA in stage A2 prostate cancer which correlated with disease progression. Okano<sup>174</sup> also found that patients with poorly differentiated carcinoma, exhibiting no PSA staining, had a poorer prognosis.

## RECENT ADVANCES IN THE APPLICATION OF PSA TO THE DIAGNOSIS AND SCREENING OF PROSTATE CANCER

Various attempts have been made to improve the detection rate of prostate cancer, especially in its early stages. Serum PSA levels in asymptomatic healthy males have been found to correlate with prostate volume and age.<sup>175-179</sup> Labrie et al.<sup>176</sup> have found a correlation coefficient of  $r = 0.184$  in benign cases and  $r = 0.398$  in prostate cancer. Therefore, the "normal value" of PSA changes with age (Age-specific PSA reference) and the degree of change of serum PSA may predict the occurrence of prostate cancer (PSA velocity).<sup>175</sup> Furthermore, following Babarian's observations,<sup>177</sup> Benson et al.<sup>180-181</sup> evaluated serum PSA levels divided by prostatic volume (PSA density, PSAD) for the differential diagnosis of prostate cancer

Table 6. Clinical usefulness of tumor markers in the follow-up of prostate cancer patients.

Tumor marker & detection methods	PSA								PAP	
	Tandem-R	Markit-F	Markit-M	Eiken	Pros-Check	Delfia	CIS	ACS	$\gamma$ -Sm	
<b>Responder</b>										
No of cases	30	10	9	28	10	10	10	5	29	30
Cases score $\geq 3$	30	10	9	26	10	10	10	5	18	17
(%)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(62)	(57)
Mean score	3.5	3.9	3.8	3.8	3.4	3.4	3.4	3.4	3.0	3.0
<b>Non-responder</b>										
No of cases	13	5	4	13	3	5	4	4	12	12
Cases score $\geq 3$	10	5	4	10	2	5	4	4	8	7
(%)	(77)	(100)	(100)	(77)	(67)	(100)	(100)	(100)	(67)	(58)
Mean score	2.9	3.2	3.3	2.8	3.0	3.2	3.3	3.3	2.5	2.3
<b>Total</b>										
No of cases	43	15	13	41	13	15	14	9	41	42
Cases score $\geq 3$	40	15	13	36	12	15	14	9	28	24
(%)	(93)	(100)	(100)	(88)	(92)	(100)	(100)	(100)	(68)	(59)
Mean score	3.3	3.7	3.6	3.3	3.3	3.3	3.4	2.9	2.9	2.8

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Table 7. Serum PSA- $\alpha_1$ -antichymotrypsin complex levels in various diseases.

Disease	No of examination	PSA-ACT (ng/ml)		Student's t test		No of positive cases (%)		
		Range	Mean $\pm$ SD	vs NHS	vs BPH	$\geq 0.61$	$\geq 3.5$	$\geq 5.4^*$
Normal males	198	<0.10 - 1.0	0.182 $\pm$ 0.140	-	-	3 (1.5)	0	0
Normal females	19	<0.10 - 0.50	0.189 $\pm$ 0.129	NS	-	0	0	0
RCC/BT	60	<0.10 - 3.4	0.600 $\pm$ 0.741	<0.0001	-	15 (25)	0	0
Acute prostatitis	4	1.6 - 3.2	2.40 $\pm$ 0.616	<0.0001	NS	4 (100)	0	0
BPH	91	<0.10 - 10	1.48 $\pm$ 1.95	<0.0001	-	55 (60)	9 (10)	6 (7)
Prostate cancer	79	<0.10 - 4200	163 $\pm$ 569	<0.0001	<0.01	68 (86)	55 (70)	54 (68)
Stage A	12	<0.10 - 82	9.34 $\pm$ 26.2	<0.0001	<0.005	7 (58)	2 (17)	2 (17)
Stage B	13	0.30 - 48	11.5 $\pm$ 18.4	<0.0001	<0.0001	11 (85)	5 (38)	5 (38)
Stage C	18	<0.10 - 410	71.9 $\pm$ 115	<0.0001	<0.0001	16 (89)	15 (83)	15 (83)
Stage D	36	0.10 - 4200	314 $\pm$ 618	<0.0001	<0.0005	34 (94)	33 (92)	32 (89)
WEL	13	<0.10 - 310	33.6 $\pm$ 84.1	<0.0001	<0.0005	-	-	-
MOD	17	0.20 - 470	63.1 $\pm$ 143	<0.0001	<0.0001	-	-	-
POR	18	1.2 - 2800	233 $\pm$ 642	<0.0001	<0.001	-	-	-

\* 0.61 ng/ml (mean + 3SD) in normal males and 3.5 and 5.4 ng/ml (mean + SD and + 2SD) in BPH patients respectively.

Table 8. Characteristics of assays for measuring serum PSA levels.

PSA assays	Markit-F PA	Eden PSA	Tandem-R PSA	Prog-Check PSA	Deflia PSA	Ball ELSA PSA	ACS PSA	Markit-M PA
Produced in	Japan	Japan	USA	USA	Sweden	France	USA	Japan
Principle	EIA competition	RIA double-antibody	IRMA sandwich	RIA double-antibody	TRFIA sandwich	IRMA one-step sandwich	CLIA one-step sandwich	EIA one-step sandwich
1st Ab	PoAb	PoAb	MoAb	PoAb	MoAb	MoAb	MoAb	MoAb
2nd Ab	anti rabbit-IgG	anti rabbit-IgG	MoAb	anti rabbit-IgG	MoAb	MoAb	MoAb	MoAb
Tracer	$\beta$ -Gal	$^{125}$ I	$^{125}$ I	$^{125}$ I	Eu	$^{125}$ I	CL	HRP
Detection range (ng/ml)	1.5-300	1.0-100	0.2-100	0.5-50	0.1-500	0.2-130	0.13-260	0.1-100

and BPH. There was a statistically significant difference in PSAD between BPH and prostate cancer. This observation was confirmed by Lee et al.<sup>182</sup> As a critical cut-off point for PSAD, a value of 0.20 is recommended. Further work in this area is now being carried out, especially in Japan.

As PSA is a serine protease,<sup>32-35,183</sup> serum PSA must be conjugated to a protease inhibitor for neutralization. Stenman et al.<sup>184</sup> and Lilja et al.<sup>185</sup> independently found that PSA reacts with  $\alpha_1$ -antichymotrypsin (ACT) in serum and the resulting complex is the major form in which PSA is present in the serum of patients with prostate cancer. The same groups<sup>184-185</sup> and Wood et al.<sup>186</sup> have established immunoassays for the determination of the PSA-ACT complex and evaluated its use in differentiating prostate cancer from BPH. Their results have prompted a search for a specific indicator for prostate cancer. In Japan, Kuriyama et al.<sup>187</sup> have developed an RIA for PSA-ACT and are evaluating its clinical significance.

Preliminary results indicate that serum PSA-ACT levels, even in stage A prostate cancer, are significantly higher than in BPH (Table 7). In addition, Christensson et al.<sup>188</sup> have used a specific monoclonal antibody and reported that free PSA is a more sensitive indicator of prostate cancer. The assay kit for  $\gamma$ -Sm detects only the free form of PSA<sup>188</sup> and, therefore, Demura et al.<sup>189</sup> have evaluated the PSA/ $\gamma$ -Sm ratio for the detection of prostate cancer. They have recommended a PSA/ $\gamma$ -Sm ratio of 1.45 as the most effective cut-off point for differentiating between prostate cancer and BPH. Thus,  $\gamma$ -Sm is now being reevaluated for assaying the free form of PSA.

#### STANDARDIZATION OF PSA ASSAYS

As mentioned above, there are more than 10 assay kits available in Japan. In Table 8, details of 8 are summarized. Each of them has different characteristics such as the sensitivity of detection, working range, antibody

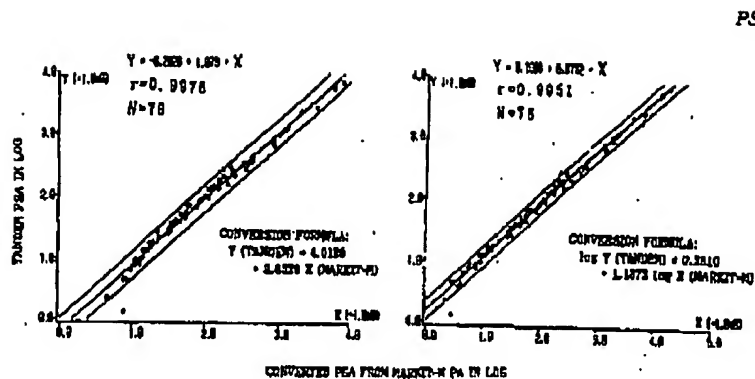


Fig. 2. Conversion between PSA assays, from Markit-M PA to the Tandem-R PSA in prostate cancer cases.

used (whether monoclonal or polyclonal), antigen determining site, RIA or non-isotopic method, and tracer used. Not surprisingly, this tends to confuse urologists when analyzing the data. The difficulty in comparing data from the Tandem-R PSA and Pro-Check PSA assays has been described by Graves et al.<sup>191</sup> They pointed out the need for an international PSA standard as early as 1990.<sup>192</sup> To this end, Stanczy organized an International Conference for PSA Standardization at Stanford in 1992 which recommended the p30 of Sepsabaugh<sup>95</sup> as the world PSA standard because p30 has been shown to be identical to PSA.<sup>96</sup> A second conference is scheduled at Stanford for Summer 1994.

In another attempt to standardize PSA assays, The Japanese Urological Association (JUA) set up a sub-committee to examine the possibility of comparing data in Japanese studies.<sup>192</sup> Using 142 sera from BPH and prostate cancer cases, the group successfully produced formulae for comparison using a linear regression technique. These conversion formulae were then evaluated in practice. Serum PSA levels were simultaneously measured by Tandem PSA and Markit-M PA (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) or Eiken PSA kits in patients with BPH and prostate cancer; only data above the limit of detection were used in this comparison. High correlations between the measured and converted Tandem-R PSA values were observed. Correlation coefficients ranged from 0.8758 in the case of the Eiken PSA kit converted using a logarithmic scale in the BPH cases ( $n = 58$ ) to 0.9978 for the Markit-M PA kit in prostate cancer cases ( $n = 76$ ). The 95% of confidence limits of the linear regression curves were very narrow in the case of the Markit-M PA kit for BPH ( $n = 63$ ) and prostate cancer (Fig. 2) and for the Eiken PSA kit in prostate cancer patients ( $n = 101$ ). These data suggest that the converting formulae proposed by the JUA for the standardization of various PSA assays have a practical use.

## CONCLUSIONS

PSA is a glycoprotein with a molecular weight of 33,000–34,000 and it has a kallikrein-like, serine protease activity. It is exclusively produced by the epithelial cells of the prostate, regardless of tissue type, and hence can be regarded as a prostate tissue-specific protein. Approximately 25–75% of patients with BPH have elevated serum PSA levels depending upon the type of assay kit or normal range used. Therefore, it is unlikely that PSA by itself will become an effective screening tool for the diagnosis of the early stages of prostate cancer. However, by using a recently developed assay method involving PSAD, PSA-ACT complex and the free form of PSA, it may be possible to make a differential diagnosis between prostate cancer and BPH. PSA is an extremely sensitive tumor marker for monitoring patients after therapy.

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